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Abstract: Glutamine synthetase (GS) is the enzyme responsible for the biosynthesis of glutamine, providing the only source of endogenous glutamine necessary for several critical metabolic and developmental pathways. GS deficiency, caused by pathogenic variants in the glutamate-ammonia ligase (GLUL) gene, is a rare autosomal recessive inborn error of metabolism characterized by systemic glutamine deficiency, persistent moderate hyperammonemia, and clinically devastating seizures and multi-organ failure shortly after birth. The four cases reported thus far were caused by homozygous GLUL missense variants. We report a case of GS deficiency caused by homozygous GLUL gene deletion, diagnosed prenatally and likely representing the most severe end of the spectrum. We expand the known phenotype of this rare condition with novel dysmorphic, radiographic and neuropathologic features identified on post-mortem examination. The biallelic deletion identified in this case also included the RNASEL gene and was associated with immune dysfunction in the fetus. This case demonstrates that total absence of the GLUL gene in humans is viable beyond the embryonic period, despite the early embryonic lethality found in GLUL animal models.

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Homozygous *GLUL* deletion is embryonically viable and leads to glutamine synthetase deficiency

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**Abstract**

Glutamine synthetase (GS) is the enzyme responsible for the biosynthesis of glutamine, providing the only source of endogenous glutamine necessary for several critical metabolic and developmental pathways. Glutamine synthetase (GS) deficiency, caused by pathogenic variants in the glutamate-ammonia ligase (*GLUL)* gene, is a rare autosomal recessive inborn error of metabolism characterized by systemic glutamine deficiency, persistent moderate hyperammonemia, and clinically devastating seizures and multi-organ failure shortly after birth. The four cases reported thus far were caused by homozygous *GLUL* missense variants. We report a case of GS deficiency caused by homozygous *GLUL* gene deletion, diagnosed prenatally and likely representing the most severe end of the spectrum. We expand the known phenotype of this rare condition with novel dysmorphic, radiographic and neuropathologic features identified on post-mortem examination. The biallelic deletion identified in this case also included the *RNASEL* gene and was associated with immune dysfunction in the fetus. This case demonstrates that total absence of the *GLUL* gene in humans is viable beyond the embryonic period, despite the early embryonic lethality found in *GLUL* animal models.

**Keywords:** Glutamine synthetase; *GLUL*; 1q25.3 deletion

**Running Title:** Homozygous *GLUL* deletion causes glutamine synthetase deficiency

## Introduction

Glutamine synthetase (GS) is the enzyme responsible for the biosynthesis of glutamine from glutamate and ammonia, providing the only source of endogenous glutamine

necessary for several essential developmental and metabolic pathways, including cellular signalling and proliferation, neurotransmitter and nucleic acid production, acid-base homeostasis, nitrogen metabolism and ammonia detoxification (1-3). GS deficiency (OMIM #610015), caused by pathogenic variants in the glutamate-ammonia ligase (*GLUL)* gene, is a rare autosomal recessive inborn error of metabolism characterized by systemic glutamine deficiency and persistent moderate hyperammonemia (4). Four cases of GS deficiency have been reported thus far (Table 1) (4-8); two presented during the neonatal period with severe encephalopathy, brain malformations and subsequent multi-organ failure and death in infancy, while the third presented with intractable seizures at 2 weeks of age and eventually died at six years of age (6). The fourth case presented at 5 months of age with seizures and developmental delay, and is still living (7). All four cases had consanguineous parents and were found to have homozygous missense variants in the *GLUL* gene (4-8). Because of the wide array of biological roles requiring glutamine and the early embryonic lethality in *GLUL* animal models (1,9,10), total absence of GS function *in utero* was thought to be embryonically lethal in humans (1). We report a case of GS deficiency caused by homozygous deletion of the entire *GLUL* gene. This case is also the first to exhibit a homozygous *RNASEL* gene deletion; while *RNASEL* mouse studies reveal a role for this gene in immune development (11), biallelic *RNASEL* deletion in humans is of unknown clinical significance. We describe the immune phenotype in the current case and suggest T cell immunodeficiency as a possible implication of biallelic *RNASEL* deletion.

**Case description**

A 28-year-old woman of Czech descent was evaluated in the Prenatal Genetics clinic for intrauterine fetal growth restriction at 15+5 weeks gestation. This *in vitro* fertilization-assisted, dizygotic dichorionic diamniotic twin pregnancy was initially uncomplicated. The woman and her partner are second cousins and had a history of male factor infertility. Family history was otherwise non-contributory.

At 15+5 weeks gestation, ultrasound revealed hydrops fetalis and intrauterine fetal demise in one twin and symmetrical growth restriction in the remaining live twin. Further ultrasounds at 17+5 weeks and 18+6 weeks gestation revealed normal anatomy in the live fetus, with persistent symmetric growth restriction. There was no evidence of placental dysfunction. At 22 and 27 weeks gestation, respectively, fetal ultrasound and brain MRI revealed microcephaly, smooth cortical mantel with delayed gyral formation, parenchymal signal changes, especially in the temporal lobes, severe vermian and cerebellar hypoplasia, thin brainstem with hypoplastic pons, and hypoplastic corpus callosum (Figure 1).

Amniocentesis was performed and microarray analysis identified a 543.6Kb homozygous 1q25.3 deletion (arr[GRCh37] 1q25.3(182058556_182601111)x0) resulting in the loss of both copies of the *GLUL* and *RNASEL* OMIM morbid genes and 6 RefSeq genes (*RGSL1, RGS16, LOC284648, JA429801, JA429802, LINC00272*). Parents were found to be heterozygous carriers of the deletion. The couple was counselled and opted for interruption of pregnancy.

*Pathological findings*

Post-mortem examination revealed a female infant at 29 weeks gestation with overall small growth parameters for gestational age (Table 1). There was a sloping forehead, hypertelorism, broad nasal bridge, bulging eyes, large tongue, thickened nuchal skin, bilateral talipes equinovarus, 2nd toe overlapping the first toe bilaterally, left 5th toe overlapping the 4th toe, and left 4th toe overlapping the 3rd toe (Figure 2). There was an ostium secundum atrial septal defect. The lungs were bilobed bilaterally. There was a persistent omphalomesenteric duct. Neuropathologic evaluation revealed microencephaly, delayed sulcation and cortical development, widespread neuronal hypoplasia, areas of abnormal cortical maturation, scattered white matter calcifications, premature germinal matrix depletion, dentate-olivary dysplasia and spinal cord hypoplasia (Figure 2). Myelination was mildly delayed. Radiologic images showed prominent coronal clefting and mild sagittal notching of the vertebral bodies, 13 pairs of ribs, disproportionate shortening of the upper and lower limbs compared to the trunk and hands, and small inferior iliac medial and lateral spurs (Figure 2). The placenta was small (<10th centile for age). Fibroblasts were obtained as a secondary source of fetal sample for genetic and metabolic testing but the cells failed to grow when cultured.

*Biochemical results*

Prior to termination of pregnancy, fetal blood was collected for amino acid quantification and revealed a very low concentration of glutamine (92 µM; neonatal reference interval (RI) 451-1,113 µM). Maternal plasma glutamine concentration was borderline low normal (379 µM; adult RI: 397-781 µM). Fetal and maternal glutamate concentrations

were normal, 83 µM (RI: 91-401 µM) and 43 µM (RI: 15-112 µM) respectively, indicating that low glutamine content was not secondary to poor specimen handling.

*Immune phenotype*

Bone marrow sample showed trilineage hematopoiesis. The thymus weight was borderline small at 1.95g (expected mean and SD for 29 weeks gestation is 3.44 +/- 1.49 g). Flow cytometry and immunohistochemistry of subpopulations in the thymus revealed $CD3^{+}CD4^{-}CD8^{-}$ cells, indicating that only very early thymocytes were detected.

**Discussion:**

Glutamine synthetase (GS) deficiency was initially characterized by neonatal epileptic encephalopathy and death from multi-organ failure in the first days to weeks of life (4). Biochemically, it is defined by decreased levels of glutamine in blood, urine and cerebrospinal fluid and chronic moderate hyperammonemia (4). Other common clinical findings are microcephaly, brain atrophy and structural brain malformations (4-6,8). The most recently described case appears to have the mildest phenotype and is the only case still living (7).

In the current case, the presence of dysmorphic features and cardiac, lung and skeletal defects broadens the known clinical phenotype of GS deficiency. Interestingly, intrauterine growth restriction was the presenting feature; growth restriction and micromelia have been described in two other cases of GS deficiency (3), suggesting a role for the *GLUL* gene in fetal growth and/or skeletal development. The most striking

neuropathological finding was the global hypoplasia affecting the brain, brainstem and spinal cord (Figure 2). This underscores the vital importance of glutamine in brain development.

Complete absence of the *GLUL* gene was previously shown to be embryonically lethal in animal models (1,9,10) and has not been reported in humans until now. In the prenatal period, glutamine is needed for cell proliferation, blastocyst implantation, brain development and overall survival (reviewed in 3), suggesting that absence of the *GLUL* gene should not be viable. Our case supports viability of homozygous *GLUL* deletion at least beyond the early embryonic period. It should be noted that the hydrops and fetal demise of the case's co-twin may be a more severe manifestation of homozygous *GLUL* deletion. Viability (until the time of interruption of pregnancy at 29 weeks gestation) in the remaining twin may have resulted from maternal glutamine released by the placenta; indeed, the mother's glutamine levels were consistent with partial enzyme production and the fetus was found to have low (92 μM), but not undetectable, levels of glutamine in her blood. Unique to this case is the fact that fetal glutamine was present presumably exclusively from maternal and/or placental synthesis as opposed to *GLUL* upregulation which was described in the other known cases (4-6,8), but would not be possible with a homozygous *GLUL* gene deletion. Our case supports previous reports of fibroblast growth failure in GS deficiency, further indicating the imperative role glutamine plays in cell survival (12).

*RNASEL gene deletion*

*RNASEL* encodes RNase L, an interferon-regulated RNA degradation enzyme which controls viral and cellular growth (11). Heterozygous and homozygous missense variants in *RNASEL* have been associated with decreased, but not absent, RNase L activity and have been linked to cancer susceptibility in humans (13,14). Immune studies on the current case showed a predominance of $CD3^{+}CD4^{-}CD8^{-}$ cells in the thymus, suggestive of a T cell immunodeficiency. The *RNASEL* deficient mouse appears to be susceptible to infections and has a defect in thymocyte apoptosis (11), which is inconsistent with our findings. This could be due to species-specific diversity or that the findings in the immune system of the current case result from a combined effect of deficiency in *RNASEL* and one or more of the other genes in the deletion.

As with all copy number variations affecting more than one gene, it is difficult to distinguish how each gene involved contributes to the overall phenotype. A review of the 6 RefSeq genes (*RGSL1, RGS16, LOC284648, JA429801, JA429802, LINC00272*) affected by the deletion in addition to *GLUL* and *RNASEL*, and the areas of homozygosity apparent on the microarray, did not identify genes that would better explain the current case's phenotype. Therefore, we propose that biallelic loss of *RNASEL* function may be a novel cause of T cell immunodeficiency. Alternatively, because glutamine is utilized in T lymphocyte activation (15), immune dysfunction may be an unrecognized feature of GS deficiency and immunological testing may be warranted in these cases. Further studies on the immune function in GS deficiency patients and the role of *RNASEL* in immune development are needed to clarify this

association. Until then, we propose that children diagnosed with GS deficiency undergo immune evaluation to identify potentially treatable deficiencies.

Here, we present a case of GS deficiency caused by total absence of the *GLUL* gene, likely representing the most severe end of the spectrum. The novel findings presented here should prompt investigations for cardiac, lung and skeletal malformations in current and future cases of GS deficiency. Maternal supply of glutamine appears to support fetal viability in the context of homozygous *GLUL* deletion in the fetus. Further studies are needed to identify whether glutamine supplementation in carrier mothers may ameliorate or even prevent the severe multisystemic sequelae of this rare condition.

**References:**


1. He Y, Hakvoort TBM, Vermeulen JLM, Lamers WH, Van Roon MA. Glutamine synthetase is essential in early mouse embryogenesis. Developmental dynamics. 2007;236:1865-1875.
2. Fumarola C, Zerbini A, Guidotti GG. Glutamine deprivation-mediated cell shrinkage induces ligand-independent CD95 receptor signalling and apoptosis. Cell Death Differ. 2001;8:1004-13.
3. Spodenkiewicz M, Diez-Fernandez C, Rufenacht V, Gemperle-Britschgi C, Haberle J. Minireview on Glutamine synthetase deficiency, an ultra-rare inborn error of amino acid biosynthesis. Biology. 2016; 5, 40.

4. Haberle J, Gorg B, Rutsch F, Schmidt E, Toutain A, Benoist JF, Gelot A, Suc AL, Hohne W, Schliess F, Haussinger D, Koch HG. Congenital glutamine deficiency with glutamine synthetase mutations. NEJM. 2005. 353:1926-33.
5. Haberle J, Gorg B, Toutain A, Rutsch F, Benoist JF, Gelot A, Suc AL, Koch HG, Schliess F, Haussinger D. Inborn error of amino acid synthesis: Human glutamine synthetase deficiency. J Inherit Metab Dis 2006. 29:352-358.
6. Haberle J, Shahbeck N, Ibrahim K, Hoffmann GF, Ben-Omran T. Natural course of glutamine synthetase deficiency in a 3 year old patient. Molecular Genetics and Metabolism. 2011. 103:89-91.
7. Unal O, Ceylaner S, Akin R. A very rare etiology of hypotonia and seizures: congenital glutamine synthetase deficiency. Neuropediatrics. 2019. 50:51-53
8. Haberle J, Shahbeck N, Ibrahim K, Schmitt B, Scheer I, O-Gorman R, Chaudhry FA, Ben-Omran T. Glutamine supplementation in a child with inherited GS deficiency improves the clinical status and partially corrects the peripheral and central amino acid imbalance. Orphanet J Rare Dis. 2012. 25;7:48.
9. He Y, Hakvoort TBM, Vermeulen JLM, Labruyere WT, Waart RD, Van Der Hel WS, Ruijter JM, Uylings HBM, Lamers WH. Glutamine synthetase deficiency in murine astrocytes results in neonatal death. Glia. 2010. 58:741-754.
10. Cagesse C, Caizzi R, Barsanti P, Bozzetti MP. Mutations in the glutamine synthetase I (gsI) gene produce embryo-lethal female sterility in Drosophila melanogaster. Dev Genet. 1992;13(5)359-66
11. Zhou A, Paranjape J, Brown TL, Nei H, Naik S, Dong B, Chang A, Trapp B, Fairchild R, Colmenares C, Silverman RH. Interferon action and apoptosis are

defective in mice devoid of 2-prime,5-prime-oligoadenylate-dependent RNase L.The EMBO Journal. 1997. 16;21:6355-6363.

12. Vermeulen T, Gorg B, Vogl T, Wolf M, Varga G, Toutain A, Pail R, Schliess F, Haussinger D, Haberle J. Arch Biochem Biophys. 2008;478(1):96-102.
13. Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Grönberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet. 2002 Feb;30(2):181-4.
14. Madsen BE, Ramos EM, Boulard M, Duda K, Overgaard J, Nordsmark M, Wiuf C, Hansen LL. Germline mutations in RNASEL predicts increased risk of head and neck, uterine, cervix and breast cancer. PLOS One 2008. 3;6:e2492.
15. Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, Turay AM, Frauwirth KA. Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. J Immunol. 2010, 185:1037-1044.

**Figure Legends**

**Figure 1: Fetal imaging studies in a case of homozygous 1q25.3 deletion.**

*Ultrasound images (top)* revealed short femur (A) at 22 weeks gestation, cerebellar hypoplasia (arrow), delayed gyral formation and absent cavum septum pellucidum (*) at 22 weeks (B) and 26 weeks (C,D) gestation.

*Fetal MRI at 27 weeks' gestation (bottom):* Single shot fast spin echo (SSFSE) images reveal severely hypoplastic corpus callosum (G; short arrow), thin brainstem with hypoplastic pons (G; arrow) vermian hypoplasia and multiple periventricular cavitations (E; arrow), severely hypoplastic cerebellum (arrow), and marked simplification of cortical gyral development (arrow) (H).

**Figure 2: Post-mortem findings in a case of homozygous 1q25.3 deletion.**

Radiographic images show upper and lower limbs disproportionately short compared to trunk and hands (A), prominent coronal clefting (white arrow), sagittal notching (black arrow) of vertebral bodies (B), small inferior iliac medial and lateral spurs (C).

Gross pathological examination revealed microencephaly with delayed sulcation and cortical development (D) compared to typical brain at same gestational age (E) and facial dysmorphisms (F). Coronal view of brain anatomy (G) showed overall preservation of general architecture with extreme atrophy and premature germinal matrix depletion, hypoplastic corpus callosum with irregular aspect anteriorly (*), subependymal zone rarified with germinolysis (arrow). Histopathology demonstrating overall cortical architecture preserved (H), prominent laminar arrangement with excess of microlaminar within the developing cortical laminae (I).